Support for the new limitation that the acetylation status consists essentially of NAD-dependent acetylation status can be found in the specification at page 55, line 26 through page 56, line 3. As described therein, lysine at position 16, deacetylation of which by Sir2 is NAD-dependent, was deacetylated by the presence of Sir2 and NAD, while other residues of the same peptide, namely lysine 5, lysine 8, and lysine 12, deacetylation of which is not NAD-dependent, were poor substrates for Sir2 activity. Therefore, Applicants have demonstrated a method of altering acetylation status of at least one amino acid residue in a protein (in this case a histone protein), wherein the acetylation status consists essentially of NAD-dependent acetylation status, by combination with a Sir2 protein; the activity of the Sir2 protein was specific to NAD-dependent deacetylation.

Claims 11, 21 and 25 have also been amended to more clearly define and particularly point out that NAD or an NAD-like compound are used in the methods. Support for the amendment is found, for example, on page 3, line 10 through page 4, line 4; page 4, line 28 through page 5, line 2; and page 16, lines 11-20.

Amended Claims 2 and 26 and new Claims 63, 64 and 67 include a limitation that the protein is a histone protein. Support for the claim amendments can be found in the specification, for example, page 2, lines 23-25; page 4, line 28 through page 5, line 2; page 4, lines 5-16; page 4, lines 5-16; and page 3, line 22 through page 4, line 4.

New Claim 65 is directed to a method of altering a life span of a cell by administering to the cell an agent which alters acetylation status of at least one amino acid in a protein, the acetylation status consisting essentially of NAD-dependent acetylation status by altering the activity of a Sir2 protein. The agent used in the method of Claim 65 is identified by a method comprising the steps of combining the protein, a Sir2 protein, NAD or an NAD-like compound and detecting a difference in the acetylation status of the amino acid of the protein in the presence of the agent compared to the acetylation status in the absence of the agent. Support for new Claim 65 can be found throughout the specification. For example, page 3, line 10 through page 4, line 4; page 17, line 22 through page 18, line 9; and page 16, lines 11-26. Support for the alteration of an amino acid in a protein deacetylation status consisting essentially of NAD-dependent acetylation is as described for Claims 11, 21 and 25.

New Claims 62 and 66 include a limitation that the histone protein is a histone protein selected from the group consisting of an H2B, H3 and H4 histone protein. Support for a new Claims 62 and 66 can be found in the specification, for example, page 2, lines 25-26; page 16, line 27 through page 17, line 1; and page 17, lines 19-21.

No new matter has been added in the claim amendments or new Claims 62-67. Entry is respectfully requested.

Rejection of Claims 1-27 Under 35 U.S.C. §112, First Paragraph

Claims 1-27 are rejected under 35 U.S.C. §112, first paragraph as containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to make and use the invention. The Examiner stated that the specification failed to provide guidance as to what comprises an "acetylation status," which proteins are affected by "acetylation status," the difference between "acetylation status" and "NAD-dependent acetylation status," how acetylation status is altered or determined and what role histone proteins or Sir2 proteins have in the claimed methods. The Examiner further stated that the specification fails to provide guidance as to which acetyl group of a protein is removed.

Applicants have defined "acetylation status" throughout the specification. For example, on page 16, lines 11-12 and lines 21-26, "acetylation status" and "NAD-dependent acetylation is defined as:

> "NAD-dependent" as used herein refers to a requirement for NAD (nicotinamide adenine dinucleotide) compound in a reaction. . . .
>
> The term "NAD-dependent acetylation status" refers to the requirement of NAD to either transfer (also referred to herein as the addition) or remove (also referred to herein as deacetylation) at least one acetyl group (e.g., CH_3CO--) to a substrate having OH or NH_2 groups (e.g., at least one amino acid residue of a histone protein such as H2B, H2A, H3 and/or H4). Thus, "acetylation status" can be either acetylation or deacetylation of a substrate.

Therefore, the "acetylation status" is the presence or absence of an acetyl group of an amino acid residue of a protein, such as the acetyl group on the lysine residue of a histone protein. When the acetylation or deactylation of an amino acid residue of a protein is dependent upon the presence of NAD, the "acetylation status" is an "NAD-dependent acetylation status."

The specification describes alterations in the acetylation status of proteins, including histone proteins. See, for example, page 2, lines 7-9; page 15, line 24 through page 16, line 2; page 16, lines 5-10; page 16, line 27 through page 17, line 6; page 76, line 4 through page 77, line 10; page 79, line 7 through page 80, line 16 of the specification.

Contrary to the Examiner's statement, the specification provides guidance on how to alter acetylation status and how to determine acetylation status. Acetylation status of a peptide, in particular, a histone peptide, is altered by Sir2, as described on page 38, lines 1-11; page 55, line 15 through 56, line 8; page 79, line 7 through page 84, line 17; Figures 8a-8g, 9a-9c, 10a-10f, 11a-11d, 12c, 13a-13e, 15a-15h and 16a-16d.

Acetylation status of a protein can be determined using methods known to one of skill in the art. Methods such as electron-spray or matrix assisted laser desorption/ionization (MALDI) mass spectroscopy are described in the specification, for example, on page 17, lines 25 through page 18, line 2; page 18, lines 12-15; page 69, line 12 through page 70, line 27; page 76, line 4 through page 77, line 10; page 79, line 7 through page 81, line 22. In addition, Claim 20 is directed to a method of detecting NAD-dependent acetylation status of a protein using electron-spray mass spectroscopy.

As discussed above, the specification provides guidance as to the role of Sir2 and histone proteins in Applicants' claimed methods. Applicants' claimed invention, as amended, is generally directed to methods of altering the acetylation status of at least one amino acid residue in a protein by altering the activity of Sir2, the acetylation status consisting essentially of NAD-dependent acetylation status. Chromatin proteins, in particular, histone proteins, more specifically, lysine residues of H3, can be deacetylated by Sir2 as described, for example, at page 55, line 15 through page 57, line 8; page 58, line 1; page 59, line 7; page 79, line 7 through page 80, line 17; and page 82, line 16 through page 87, line 4.

Contrary to the Examiner's statement, there is guidance in the specification as to the role of Sir2 proteins in the removal of acetyl groups. For example, as described on page 17, lines 3-6,

any suitable amino acid having an -OH or -NH_2 group, is capable of undergoing an alteration in acetylation. In particular, as described on page 17, lines 2-3 an acetyl group on lysine 9 or lysine 14 of H3; or lysine 16 of H4 can be removed by combination with Sir2. Deacetylation is defined, on page 20, lines 20-21, as removal of an acetyl group from at least one amino acid which has an NH_2 group.

Therefore, Claims 1-27 are described in the specification in such a way as to enable one to make and use the invention. The specification provides guidance as to what "acetylation status" and "NAD-dependent acetylation status" are; how acetylation status is determined; and the role of Sir2 in the claimed methods.

Rejection of Claims 1-27 Under 35 U.S.C. §112, Second Paragraph

Claims 1-27 are rejected under 35 U.S.C. §112, second paragraph as being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicants regard as the invention. The Examiner stated the terms "altering the acetylation status" and "acetylation status" are indefinite. The Examiner further stated that it is unclear by what method acetylation status is determined and the claims are indefinite for failing to define what criteria is used to determine acetylation status.

As discussed above, the term "acetylation status" and methods to determine the acetylation status are defined in the specification and, thus, meet the requirements of 35 U.S.C. § 112, second paragraph.

The Examiner also stated that Claims 1, 6, 11, 21, 24 and 25 are vague and indefinite by use of the term "NAD-dependent acetylation status." The Examiner further stated that it is not clear how NAD-dependent acetylation is determined and how NAD-dependent acetylation differs from "acetylation status."

As discussed above, the specification describes the meaning of "NAD-dependent acetylation" and the difference with "acetylation status." Thus, the term "NAD-dependent acetylation" are clear and definite. In addition, as discussed above, the specification describes how NAD-dependent acetylation status is determined, including determination by electron-spray mass spectroscopy as recited in Claim 20.

The Examiner further stated, with regard to Claim 1, that it was unclear how "acetylation status consists essentially of NAD-dependent acetylation status." Applicants noted that the claim employs the phrase "consisting essentially of NAD-dependent acetylation status."

As stated in the Manual of Patent Examining Procedure (MPEP) at § 2111.03, page 2100-50, transitional phrases such as "consisting essentially of" can be used to define the scope of a claim with respect to what unrecited additional components or steps, if any, are excluded from the scope of the claim. Specifically the MPEP states:

> The transitional phrase "consisting essentially of" limits the scope of a claim to the specified materials or steps "and those that do not materially affect the basic and novel characteristic(s)" of the claimed invention. "A 'consisting essentially of' claim occupies a middle ground between closed claims that are written in a 'consisting of' format and fully open claims that are drafted in a 'comprising' format." (emphasis in original) (citations omitted).

Thus, the use of the transition phrase "consisting essentially of" does not render the claim vague and indefinite.

Examiner further stated that Claim 1 was vague and indefinite because of the use of the phrase "altering the activity of **a** Sir2 protein" (emphasis in Office Action). The Examiner stated it was unclear whether "**a**" refers to a single protein molecule or a species. The Examiner further stated that the method of Claim 1 was vague and indefinite for failure to recite a positive active step in the process.

Applicants have amended Claim 1 to recite an active to step to define the process. Contrary to the Examiner's statement, the phrase "altering the activity of a Sir2 protein" is definite and clear. As described in the specification, a Sir2 protein has deacetylase activity (see, for example, page 38, lines 1-11). Mutations in the Sir2 protein can deactivate the deacetylase activity of Sir2 (see for example, page 82, line 16 through page 83, line 4). Thus, "altering the activity of a Sir2 protein" is defined and used with clear meaning.

The term "Sir2 protein" is defined in the specification on page 36, lines 6-11 and refers to the protein product of a Sir2 gene. Thus, "a Sir2 protein" is definite.

The Examiner stated that the limitation "the histone protein" in Claim 2 lacked antecedent basis. Applicants have amended Claim 2 to include antecedent basis for a histone protein.

The Examiner further stated that Claim 6 and 14 were vague and indefinite for use of the phrase "removal of an acetyl group." The Examiner stated it was unclear how the acetyl group was removed, what the acetyl group was removed from, what role Sir2 played in removal of the acety group and whether removal of the acetyl group is the activity of Sir2.

Claims 6 and 14 are dependent claims of the methods of Claims 1 and 11, respectively, wherein the alteration in the NAD-dependent acetylation status of at least one amino acid residue in the protein is removal of an acetyl group. In other words, Sir2 removes, in an NAD-dependent manner, an acetyl group from at least one amino acid of a protein. The acetyl group is removed from the acetylated protein by Sir2. As discussed above, the phrase "NAD-dependent acetylation status" is defined, on page 16, lines 21-25, in the specification and refers to a requirement of NAD to either add (acetylate) or remove (deacetylate) at least one acetyl group (e.g., CH_3 CO--) to or from, respectively, a substrate having an -OH or -NH_2 group. Thus, "removal of an acetyl group," referred to in Claims 6 and 14, is defined as removal of "at least one acetyl group," e.g., "CH_3 CO--" on at least one amino acid residue in a protein. Thus, "removal of the acetyl group" is defined and Claims 6 and 14 are clear and definite.

The Examiner stated that Claims 8, 10, 15, 22, and 26 recite improper Markush language. Applicants have amended Claim 8, 10, 15, 22 and 26, thereby obviating the rejection.

The Examiner stated that Claim 11 was vague and indefinite for use of the term "NAD compound." Applicants have amended Claim 11, thereby obviating the rejection. Applicants have also amended Claims 21 and 25 to more clearly define the NAD used in the methods of Applicants' claimed invention.

The Examiner further stated that Claims 11 and 21 are vague and indefinite for use of the phrase "detecting the NAD-dependent status." The Examiner stated that it was impossible to determine the metes and bounds of the invention because it cannot determine how NAD-dependent status was detected.

As discussed above, methods to detect NAD-dependent acetylation status are described in the specification and are known to one of skill in the art. For example, as described on page 17,

lines 27-29, NAD-dependent acetylation status can be detected by electron-spray or matrix assisted laser desorption ionization (MALDI) mass spectroscopy. Detection of differences, by electron-spray mass spectroscopy, in a NAD-dependent acetylation status are shown in, for example, Figures 9a-9d and described on page 70, lines 14-27; page 79 line 16 through page 80, line 17. Thus, Claims 11 and 12 are clear and definite by use of the term "detecting the NAD-dependent status." Furthermore, Claim 20, which depends on Claim 11, is directed to a detection step performed by electron-spray mass spectroscopy.

The Examiner stated that Claims 11 and 21 are vague and indefinite for use of the phrase "producing a combination." The Examiner stated that it was unclear what was meant by "a combination" or how a combination is "produced."

The meaning of the term "combination" is clear; the combination of the step of the method of Claims 11 and 21, as amended, is produced by combining a protein, a Sir2 protein, NAD or an NAD-like compound and an agent to be tested. Thus, a "combination" is formed when the protein, the Sir2 protein, NAD or an NAD-like compound and the agent to be tested are combined, such as by mixing suspensions of the respective components. When combined, a "combination" is produced. Therefore, Claims 11 and 21 meet the requirements of 35 U.S.C. § 112, second paragraph.

The Examiner stated that Claims 12 and 13 are vague and indefinite because of the phrase "activity of the Sir2 protein."

Claims 12 and 13 depend on the method of Claim 11, which is a method of identifying an agent which alters the activity of a Sir2 protein by assessing the ability of the agent to alter the NAD-dependent acetylation status of at least one amino acid in a protein. The activity of Sir2 is the ability of Sir2 to alter NAD-dependent acetylation status of a protein, which is defined, for example, on page 15, lines 25-26; page 29, lines 1-15; page 70, line 1-27; page 79, line 7 through page 83, line 4. In particular, page 82, line 16 through page 83, line 6 and Figures 15, 17a and 17b show that mutations in Sir2 result in a decrease in the deacetylase activity of Sir2 activity. Specifically, Sir2 activity is defined on page 38, lines 1-11:

> The "biological activity" of a Sir2 protein is defined herein to mean the ability to alter the NAD-dependent acetylation status of a

> histone protein and/or the mono-ADP-ribosylates a substrate such as a histone protein (e.g., H2B, H2A, H3, H4). In particular, the biological activity of mSir2α is the ability to alter the NAD-dependent acetylation status of lysine residues in the N-terminus of H3 (e.g., lysine 9 and/or 14) and/or H4 (e.g., lysine 16) and/or mono-ADP-ribosylate an amino acid residue of H2B, H3 or H4. Additionally, or alternatively, the biological activity also means the ability to slow aging or extend life span by, for example, repressing recombination of rDNA, or inhibiting the formation, replication and/or accumulation of rDNA circles of a cell or an organism. The biological function of Sir2 is illustrated and further defined by the Exemplification Section.

Claim 12 is directed to an agonist of Sir2 activity. An agonist is defined on page 20, line 17 through page 21, line 8:

> The term "agonist" as used herein, refers to an agent [that can] ... , for example, deacetylat[e]ing H3 or H4 (e.g., removal of an acetyl group from at least one lysine residue in the amino terminus) ... An agonist can also enhance the rate of removal of acetyl groups from amino acid residue of histone proteins.

Claim 13 is directed to agents which are antagonists of the activity of Sir2. An antagonist is defined in the specification on page 21, line 9 through page 22, line 13:

> The term "antagonist", as used herein, includes a substance [that can] ... act in a manner which prevents Sir2 from deacetylating an amino acid residue in a histone protein ... from acting on the substrate, or interferes with the accessibility of the substrate (e.g., H2B, H2 or H4) to Sir2 for alterations NAD-dependent acetylation status . . . .
>
> Alternatively or additionally, an antagonist can prevent, impede or interfere with the interaction between Sir2 and NAD ... , thereby preventing the Sir2 ... from altering the NAD-dependent acetylation status of a histone protein ... to an amino acid residue (e.g., threonine) of a substrate (e.g., a nuclear protein such as a histone protein).

The deacetylase activity of Sir2, which can be altered by agonist (Claim 12) and antagonists (Claim 13), is also shown in Figures 12a-12c and 14c, and is described in the specification, for example, page 59, lines 3-19; page, 69, lines 12-26; page, 70, lines 1-27; page 76, line 4 through page 77, line 10. Therefore, Claims 12 and 13 are clear and definite.

The Examiner stated that Claim 21 was vague and indefinite because it is unclear how a change in the acetylation status correlates to an alteration in lifespan. The Examiner further stated that Claim 21 is vague and indefinite because it is unclear how lifespan is altered.

Lifespan, in yeast, mammalian cells and an organism, is defined in the specification on page 27, line 23 through page 28, line 29. Lifespan can be altered by increasing the lifespan of an organism or decreasing the lifespan of a organism. Changes in acetylation status affect a lifespan of an organism as described, for example, on page 18, lines 2-5; page 27, lines 1-12; page 29, lines 1-15; and page 32, line 21 through page 33, line 17. Mutations in Sir2 activity that reduce or eliminate the deacetylase activity of Sir2 likewise affect lifespan of cells as described, for example, on page 59, lines 17-18 and as shown in Figure 18. Thus, Claim 21 is clear and definite.

The Examiner stated that Claim 24 was incomprehensible. The Examiner asked what method was being used to identify the agent. Applicants have cancelled Claim 24, thereby obviating the rejection.

SUMMARY AND CONCLUSION

Applicants' claimed invention meets the requirements of 35 U.S.C. §112, first and second paragraphs. Reconsideration and withdrawal of the pending rejections are respectfully request. If the Examiner believes that a telephone conference would expedite prosecution of this application, he is invited to call the undersigned at (978) 341-0036.

Respectfully submitted,

HAMILTON, BROOK, SMITH & REYNOLDS, P.C.

By Mary K. Murray

Mary K. Murray
Registration No. 47,813
Telephone: (978) 341-0036
Facsimile: (978) 341-0136

Concord, MA 01742-9133
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MARKED UP VERSION OF AMENDMENTS

Claim Amendments Under 37 C.F.R. § 1.121(c)(1)(ii)

1. (Amended) A method of altering the acetylation status of at least one amino acid residue in a protein, the acetylation status consisting essentially of an NAD-dependent acetylation status comprising the step of [by] altering the activity of a Sir2 protein.

2. (Amended) The method of Claim 1, wherein the [histone] protein is a histone protein [selected from the group consisting of an H2B, H3 or H4 histone protein].

8. (Amended) The method according to Claim 7, wherein the Sir2α protein has [the] an amino acid sequence selected from the group consisting of SEQ ID NOS: 1, 4, 9, 12, 19 [or] and 26.

10. (Amended) The method of Claim 7, wherein the Sir2α protein is a mutant Sir2α protein selected from the group consisting of G253A, G255A, S257A, I262A, F265A, R266A, G270A, P285A, T336A, H355A, Thr-261, Iso-271, Arg-275 [or] and Asn-345.

11. (Amended) A method of identifying an agent which alters the activity of a Sir2 protein by assessing the ability of the agent to alter the [NAD-dependent] acetylation status of at least one amino acid in a [histone] protein, the acetylation status consisting essentially of an NAD-dependent acetylation status, comprising the steps of:

[(]a) combining the [histone] protein, the Sir2 protein, NAD or an NAD-like compound and the agent to be tested, thereby producing a combination;

[(]b) detecting the NAD-dependent acetylation status of an amino acid in the [histone] protein in the combination; and

[(]c) comparing the NAD-dependent acetylation status of an amino acid in the [histone] protein in the combination with the NAD-dependent acetylation status of the amino acid in the [histone] protein in the absence of the agent to be tested,

wherein a difference in the NAD-dependent acetylation status of the amino acid of the [histone] protein in the presence of the agent as compared with the absence of the agent indicates that the agent alters the NAD-dependent acetylation status of at least one amino acid of the [histone] protein.

15. (Amended) The method of Claim [11] 63, wherein the histone protein is selected from the group consisting of an H2B, H3 [or] and H4 histone protein.

16. (Amended) The method of Claim [11] 63, wherein the NAD-dependent acetylation in the histone protein is acetylation of a lysine amino acid residue.

21. (Amended) A method of identifying an agent which alters life span of a cell by assessing the ability of the agent to alter the [NAD-dependent] acetylation status of at least one amino acid in a [histone] protein, the acetylation status consisting essentially of an NAD-dependent acetylation status, comprising the steps of:

[(]a) combining the [histone] protein, a Sir2 protein, [a] NAD or an NAD-like compound and the agent to be tested, thereby producing a combination;

[(]b) detecting the NAD-dependent acetylation status of an amino acid in the [histone] protein in the combination; and

[(]c) comparing the NAD-dependent acetylation status of an amino acid in the [histone] protein in the combination with the acetylation status of the amino acid in the [histone] protein in the absence of the agent to be tested,

wherein a difference in the acetylation status of the amino acid of the [histone] protein in the presence of the agent as compared with the acetylation status of the amino acid of the histone protein in the absence of the agent indicates that the agent alters the life span of the cell.

22. (Amended) The method of Claim [21] 64, wherein the histone protein is selected from the group consisting of an H2B, H3 [or] and H4 histone protein.

25. (Amended) A method of altering the [NAD-dependent] acetylation status of at least one amino acid residue in a [histone] protein, the acetylation status consisting essentially of an NAD-dependent acetylation status, comprising the step of combining the [histone] protein, a Sir2 protein and [a] NAD or an NAD-like compound.

26. (Amended) The method of Claim 25, wherein the [histone] protein is a [selected from the group consisting of an H2B, H3 or H4] histone protein.